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Address for correspondence: Federico Gobbi, Department of Infectious Tropical Diseases and Microbiology, IRCCS Sacro Cuore don Calabria Hospital, via Sempreboni 5, 37024 Negrar, Verona, Italy; email: federico.gobbi@sacrocuore.it

Helicobacter ailurogastricus in Patient with Multiple Refractory Gastric Ulcers, Japan

Masaya Sano, Emiko Rimbara, Masato Suzuki, Hidenori Matsui, Miwa Hirai, Sae Aoki, Tsuyoshi Kenri, Keigo Shibayama, Hidekazu Suzuki

Author affiliations: Tokai University School of Medicine, Kanagawa, Japan (M. Sano, M. Hirai, H. Suzuki); National Institute of Infectious Diseases, Tokyo, Japan (E. Rimbara, M. Suzuki, H. Matsui, S. Aoki, T. Kenri); Nagoya University Graduate School of Medicine, Aichi, Japan (K. Shibayama)

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We report the isolation of *Helicobacter ailurogastricus*, a *Helicobacter* species that infects cats and dogs, from a person with multiple refractory gastric ulcers. In addition to *H. suis*, which infects pigs, *Helicobacter* species that infect cats and dogs should be considered as potential gastric pathogens in humans.

A 61-year-old man in Japan had multiple ulcers diagnosed on esophagogastroduodenoscopy (EGD) performed during his annual health check-up and was referred to Tokai University Hospital (Kanagawa, Japan) because of an inadequate therapeutic response. Histologic examination of tissue from the ulcer site showed inflammatory cells and few findings suggestive of malignancy. Hematoxylin and eosin staining showed spiral bacteria resembling a *Helicobacter* species.

Test results for *H. pylori* serum antibodies and stool antigen were negative. The patient had onset of epigastric discomfort after his work became busy but attributed his symptoms to his work burden and did not seek medical care. Although he had not taken nonsteroidal antiinflammatory drugs or aspirin, he did not respond to therapy, even with the administration of the antisecretory agent vonoprazan (20 mg), and had multiple refractory gastric ulcers diagnosed.

After obtaining informed consent, we enrolled the patient in a clinical trial investigating the effects of non–*H. pylori Helicobacter* (NHPH) infections on intractable ulcers and gastric mucosa–associated lymphoid tissue lymphoma. On August 24, 2021, we assessed the patient for NHPH by using culture and PCR of gastric biopsy samples collected during EGD (Appendix, https://wwwnc.cdc.gov/EID/article/29/4/22-1807-App1.pdf). EGD showed no atrophy in the background gastric mucosa, healing of the ulcers observed previously, multiple erosions, and residual ulcers in the antrum (Figure, panel A). The PCR test result for NHPH was positive, but the bacterial culture result was negative. On November 30, 2021, a repeat EGD to assess ulcer healing status showed further healing. Repeat culture and PCR tests for NHPH were both positive. We isolated *Helicobacter* spp. strain NHP21-4376 from the greater curvature of the gastric antrum and NHP21-4377 from the lesser curvature.

The microorganisms had a corkscrew-like spiral form (Figure, panel B) resembling that of *Helicobacter suis*, the most prevalent NHPH species in the human stomach. We performed whole-genome sequencing of the NHP21-4376 and NHP21-4377 strains by using the Illumina platform (Illumina, https://www.illumina.com) (Appendix). We assembled the Illumina reads de novo by using Shovill 1.1.0 (https://github.com/tseemann/shovill) with the default parameters. We determined the bacterial species by calculating the average nucleotide identity (ANI) using pyani 0.2.12 (https://github.com/widdowquinn/pyani). Strains NHP21-4376 and NHP21-4377 had >98% identity with *H. ailurogastricus* strains, including the type strain ASB7^T, indicating that they were *H. ailurogastricus* (Appendix Figure 1).

Phylogenetic analysis based on 342 core genes among gastric *Helicobacter* species also confirmed that NHP21-4376 and NHP21-4377 are in the same clade as *H. ailurogastricus* strains ASB7^T and ASB9 and are distinct from *H. suis* strains (Appendix Figure 2). We deposited draft genome sequences of *H.*

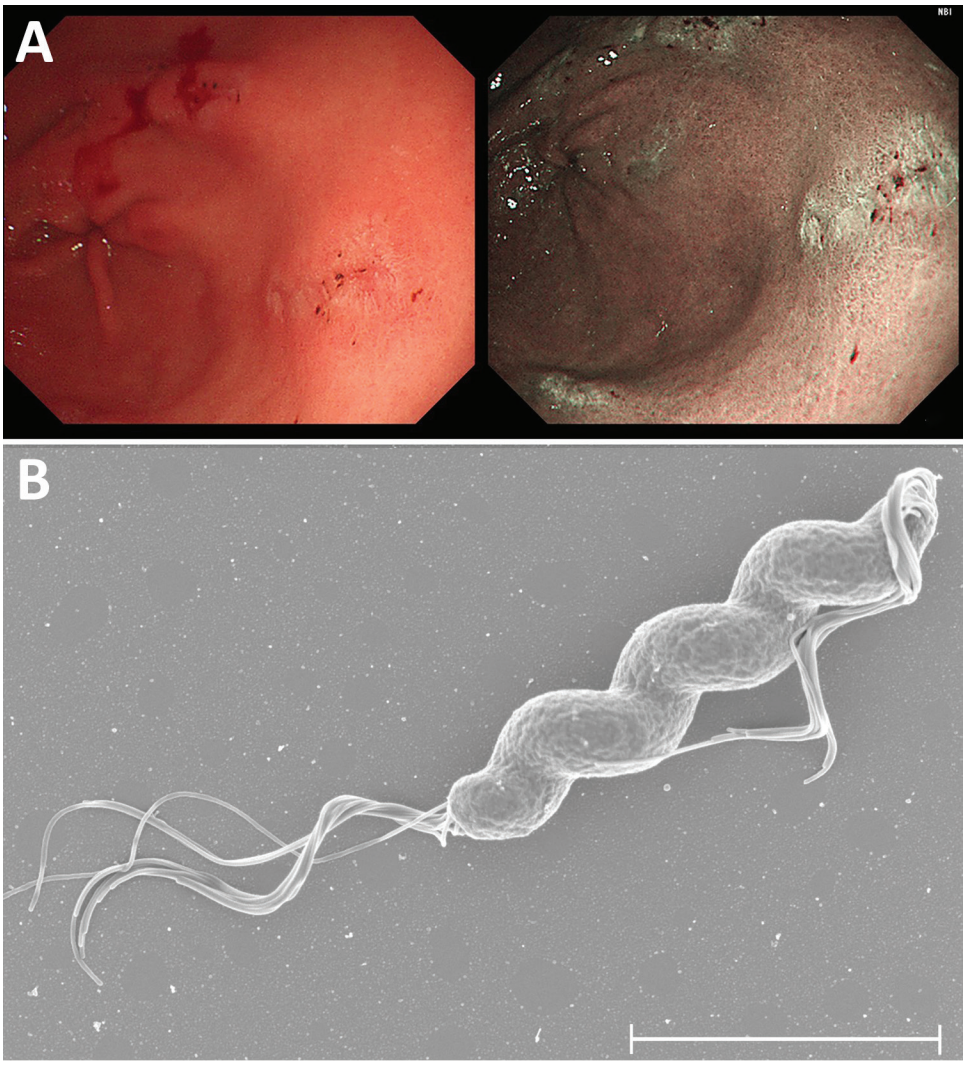

Figure. Endoscopic images from a gastric ulcer patient infected with *Helicobacter ailurogastricus* and morphologic observation and genomic comparison of isolated *H. ailurogastricus* NHP21-4376 and NHP21-4377 strains, Japan. A) Multiple linear erosions and small ulcers on the background mucosa with no evidence of atrophy in the gastric antral area. B) Scanning electron micrograph of *Helicobacter ailurogastricus* strain NHP21-4377. Scale bar indicates 2 μm.

ailurogastricus into GenBank (NHP21-4376 accession nos. BSCV01000001–64 and NHP21-4377 accession nos. BSCW01000001–66).

Antimicrobial susceptibility tests showed that *H. ailurogastricus* NHP21-4376 had a high MIC for levofloxacin (Table) and that the NHP21-4376 strain had a Ser to Arg mutation at position 78 in the quinolone resistance–determining region of DNA gyrase A (Appendix Figure 3). This position corresponds to Asn at position 87, where its mutation is responsible for fluoroquinolone resistance in *H. pylori* (*1*).

H. suis, which is the most prevalent NHPH species in humans, is believed to originate in pigs. Virulence-associated features were recently shown in *H. suis* isolates obtained from human stomachs (*2*); gastric ulcer recurrence was not observed in the patient infected with *H. suis* after *H. suis* eradication (*2*). Furthermore, *H. ailurogastricus* and *H. heilmannii* are 2 of the most prevalent NHPH strains infecting the human stomach, after *H. suis* (*3*,*4*). *H. ailurogastricus* was formerly classified as *H. heilmannii*. *H. heilmannii* and *H. ailurogastricus* are prevalent *Helicobacter* species that infect the stomachs of cats (*5*). Moreover, *H. ailurogastricus* is shown to be the prevalent gastric *Helicobacter* species infecting the stomach of cats and dogs in Japan (Appendix Table, Figure 4).

In this case, the patient was strongly suspected to have acquired the infection from his cats, although the stool of his pets could not be analyzed because the

Table. Antimicrobial susceptibilities of *Helicobacter ailurogastricus* strains ASB7^T and NHP21-4376 isolated from patient, Japan, 2021

Strain	Host	MIC, mg/L					
		Amoxicillin	Clarithromycin	Metronidazole	Minocycline	Gentamicin	Levofloxacin
ASB7^T	Cat	0.25	≤0.25	16	≤2	≤4	≤0.5
NHP21-4376	Human	1	≤0.25	16	≤2	≤4	4

patient's consent was not obtained. The patient has not had a recurrence of multiple ulcers but remains positive for *H. ailurogastricus*. The limitation of this case report is that, although we succeeded in culturing *H. ailurogastricus* in the stomach of this patient and the drug-susceptibility test has determined the regimen for eradication, we have not yet been able to perform eradication therapy. Therefore, the efficacy of eradication in *H. ailurogastricus* infections has not been confirmed. *H. ailurogastricus* eradication therapy will be administered at the next patient visit to prevent ulcer recurrence.

The clinical importance of NHPH infection in the human stomach has been increasing in the post–*H. pylori* era. Because NHPH species such as *H. suis* and *H. ailurogastricus* cannot be detected by most *H. pylori* diagnostic tests, such as the urea breath test and stool antigen test, NHPH infections should be considered when routine *H. pylori* tests are negative, despite the presence of inflammatory findings in the gastric mucosa.

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About the Author

Dr. Sano is an instructor at the Division of Gastroenterology and Hepatology, Tokai University School of Medicine. His primary research interests are endoscopic treatment of gastrointestinal diseases and elucidation of pathological mechanisms.

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Address for correspondence: Hidekazu Suzuki, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa, 259-1193, Japan; email: hsuzuki@tokai.ac.jp

Harbor Porpoise Deaths Associated with *Erysipelothrix rhusiopathiae*, the Netherlands, 2021

Lonneke L. IJsseldijk, Lineke Begeman, Birgitta Duim, Andrea Gröne, Marja J.L. Kik, Mirjam D. Klijnstra, Jan Lakemeyer, Mardik F. Leopold, Bas B. Oude Munnink, Mariel ten Doeschate, Linde van Schalkwijk, Aldert Zomer, Linda van der Graaf-van Bloois, Els M. Broens

Author affiliations: Utrecht University, Utrecht, the Netherlands (L.L. IJsseldijk, B. Duim, A. Gröne, M.J.L. Kik, J. Lakemeyer, M. ten Doeschate, L. van Schalkwijk, A. Zomer, L. van der Graaf-van Bloois, E.M. Broens); Erasmus University Medical Center, Rotterdam, the Netherlands (L. Begeman, B.B. Oude Munnink); Wageningen Food Safety Research, Wageningen, the Netherlands (M.D. Klijnstra); Wageningen Marine Research, Den Helder, the Netherlands (M.F. Leopold)

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In August 2021, a large-scale mortality event affected harbor porpoises (*Phocoena phocoena*) in the Netherlands. Pathology and ancillary testing of 22 animals indicated that the most likely cause of death was *Erysipelothrix rhusiopathiae* infection. This zoonotic agent poses a health hazard for cetaceans and possibly for persons handling cetacean carcasses.

Erysipelothrix bacteria cause infections in humans and other species after contact with infected animals or environmental sources (*1*). Illness ranges from mild to systemic, which can include septicemia and endocarditis. *Erysipelothrix* can survive for long periods in the environment, including marine ecosystems (*1*) associated with marine fish, mollusks, and crustaceans. *Erysipelothrix* infection affects captive and

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Helicobacter ailurogastricus in a Patient with Multiple Refractory Gastric Ulcers, Japan

Appendix

Methods

Non-Helicobacter pylori Helicobacter (NHPH) testing

To detect NHPH infections by PCR, DNA was extracted from the homogenates of gastric biopsy specimens using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). Then, the DNA was used as a template for probe-based real-time PCR targeting the NHPH-specific region of the 16S rRNA gene. The sequences of the two sets of primers and probes were as follows: NHPH_16S_F (5′-CAAGTCGAACGATGAAGCCTA-3′), NHPH_16S_R (5′-ATTTGGTATTAATCACCATTTCTAGT-3′), and NHPH_16S_probe (5′-/56-FAM/TTACTCACC/ZEN/CGTGCGCCACTAATC/3IABkFQ/-3′) for targeting the NHPH 16S rRNA gene. To detect NHPH infections by culture, the method for *Helicobacter suis* isolation from human gastric biopsies, as described in a previous study (*1*), was used. Briefly, the gastric biopsy specimen was homogenized in *Brucella* broth (Difco Laboratories, Detroit, MI, USA) adjusted to pH 5.0 using hydrochloric acid. The tissue homogenates were inoculated onto NHPH agar plates containing 1.5% (w/v) agar, Brucella broth, 20% (v/v) heat-inactivated fetal bovine serum, *Campylobacter*-selective supplement (Skirrow; Oxoid, Basingstoke, UK), Vitox

supplement (Oxoid), and hydrochloric acid to adjust the pH to 5.0 and incubated for more than 7 days in a humidified gas mixture (5% O_2, 12% CO_2, and 83% N_2) at 37°C. The grown colonies of the primary culture were inoculated onto NHPH agar plates and enriched by modified biphasic culture for 120 h, with shaking in a humidified gas mixture at 37°C.

Genomic Methods

Whole-genome sequencing of the *Helicobacter* spp. strains was performed using MiniSeq (Illumina, San Diego, CA, USA). The library for Illumina sequencing (150-bp paired-end; insert size, 500–900 bp) was prepared using a Nextera XT DNA Library Prep Kit. The Illumina reads were assembled de novo using Shovill v1.1.0. (https://github.com/tseemann/shovill) with the default parameters to acquire draft genome sequences. Core genome alignments among *Helicobacter* strains were determined using Roary version 3.13.0 (https://github.com/sanger-pathogens/Roary) with the default parameters. Maximum-likelihood phylogenetic trees were constructed using RAxML-NG v. 1.1 (https://github.com/amkozlov/raxml-ng) with core gene alignments. Bacterial species were determined by calculating the average nucleotide identity (ANI) using pyani 0.2.12 (https://github.com/widdowquinn/pyani).

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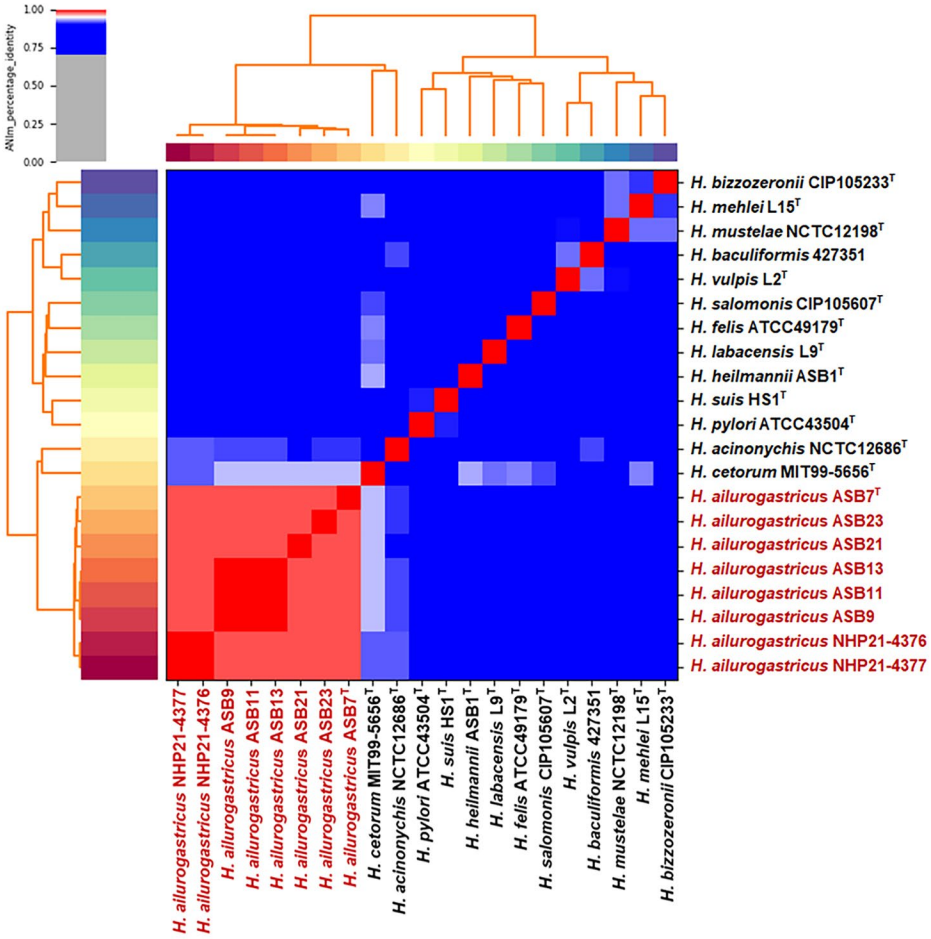
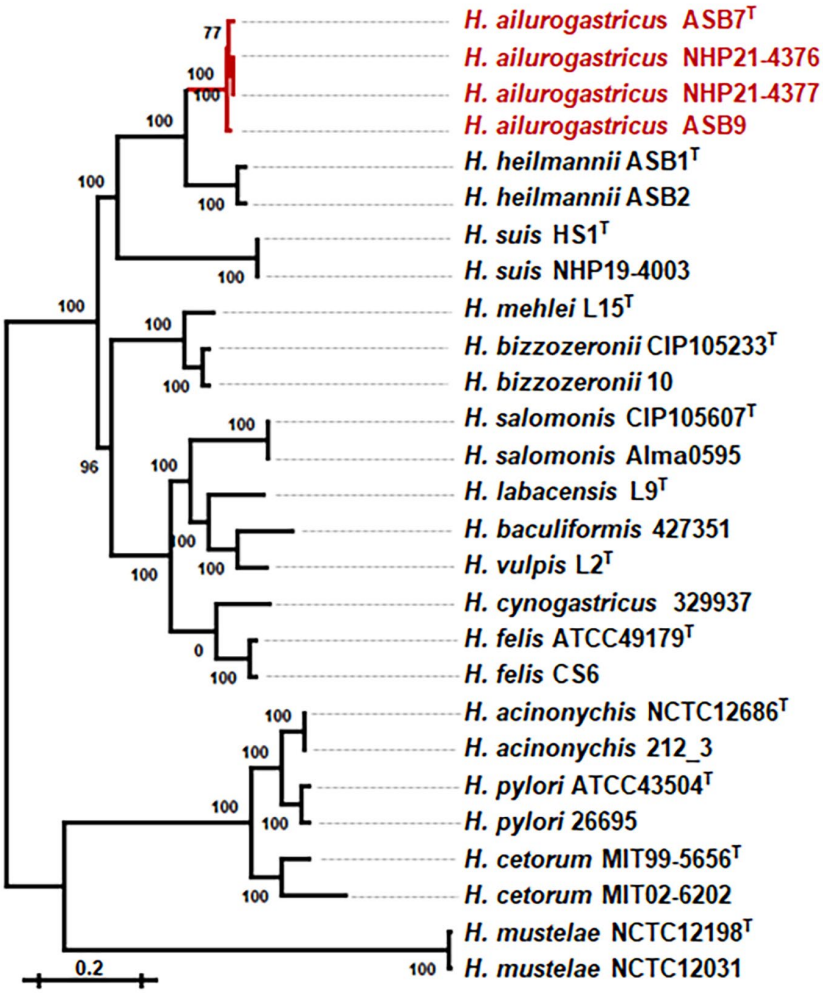
Appendix Table. Prevalence of gastric *Helicobacter* species among dogs and cats in Japan*

	No. (%) of strains					
Species	Dog (n = 47)		Cat (n = 24)		Total (n = 71)	
H. ailurogastricus	1	(2.1)	6	(25.0)	7	(9.9)
H. heilmannii	1	(2.1)	0	(0)	1	(1.4)
H. bizzozeronii	1	(2.1)	2	(8.3)	3	(4.2)
H. felis	1	(2.1)	4	(16.7)	5	(7.0)
H. pylori	1	(2.1)	0	(0)	1	(1.4)
Not classified	42	(89.4)	12	(50.0)	54	(76.1)

*The prevalence was estimated from the sequences obtained from gastric specimens of dogs (*2*) and cats (*3*) in Japan.

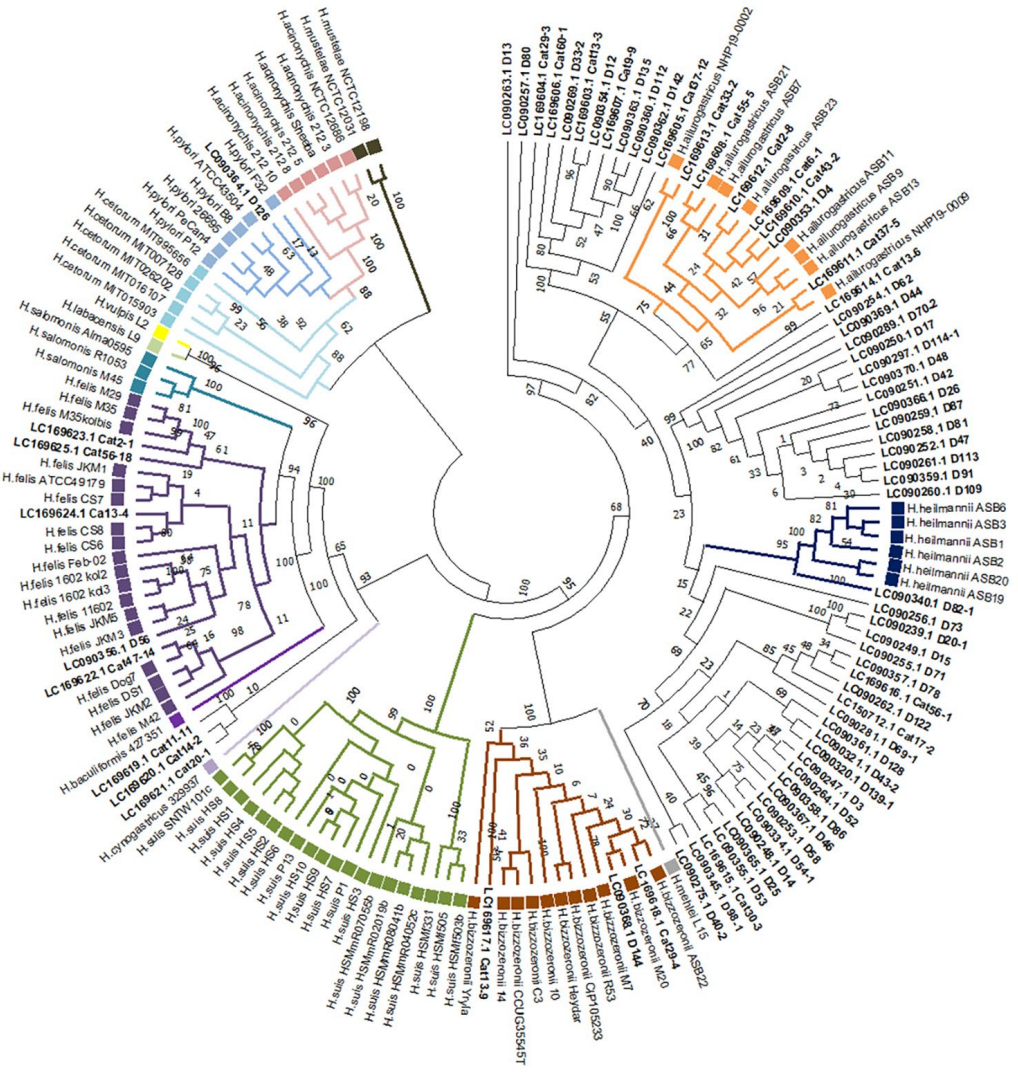


Appendix Figure 1. Average nucleotide identity among gastric *Helicobacter* species. ANI was calculated via pyani 0.2.12 using the ANI MUMmer/NUCmer method. Strains denoted in red are *H. ailurogastricus* including the NHP21–4376 and NHP21–4377 strains isolated in the study.



Appendix Figure 2. Phylogenetic tree based on 342 core genes among gastric *Helicobacter* species. Core gene alignment was constructed using Roary version 3.13.0, and the phylogenetic tree was constructed using RAxML-NG v. 1.1. The scale bar indicates the number of base substitutions per site. The lines indicate *Helicobacter ailurogastricus* strains including NHP21–4376 and NHP21–4377 obtained in this study.

Appendix Figure 3. Quinolone resistance-determining region in DNA gyrase A of *Helicobacter ailurogastricus* strains ASB7^{T} from a cat and NHP21–4376 from a human patient.



Appendix Figure 4. Phylogenetic tree generated from *ureAB* gene sequences of gastric *Helicobacter* species. The phylogenetic tree generated from the 161 *ureAB* gene sequences. The sequences were aligned by MAFFT version 7.49 and the tree was constructed using RAxML-NG version 1.1.0 with a GTR+G+I model and 100 bootstrap replicates. Numbers indicate bootstrap percentages, and the scale bar indicates the number of base substitutions per site. The *ureAB* sequences of the reference strains (squares) were extracted from whole genome sequences of gastric *Helicobacter* species obtained from NCBI. The *ureAB* sequences shown as the GenBank no. (LCXXXXXX) are obtained from cats and dogs in Japan.